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Difficulties in the Normalization of Aminotransferase Measurement with Enzyme Standards

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Summary: Commercial control sera contain varying amounts of pyridoxal-5′-phosphate. Normalization of aminotransferase activity measurement with enzyme'standards' is possible only when pyridoxal-5′-phosphate is added to the reaction medium.

Pyridoxal-5′-phosphate addition should be compulsory in national recommendations.

Schwierigkeiten bei der Kalibrierung von Bestimmungen der Aminotransferasen mit Enzymstandards

Zusammenfassung: Käufliche Kontrollseren enthalten unterschiedliche Konzentrationen von Pyridoxal-5′-phosphat. Eine Normalisierung der Aminotransferase-Aktivitätsmessung mit Enzym'standards' ist nur möglich unter Zugabe von Pyridoxal-5′-phosphat zum Reaktionsmedium.

Pyridoxal-5′-phosphat-Zugabe sollte in nationalen Empfehlungen obligatorisch sein.

Introduction

In our work on enzyme standards we encountered difficulties in the determination of aspartate aminotransferase (1, 2). In contrast to the favourable effect of enzyme standards on the results of other enzyme determinations, the use of standards conferred no benefit in the determination of aspartate aminotransferase. This seemed to be due to deficiencies in the reaction medium. In several methods and recommendations, pyridoxal-5′-phosphate is not added to the reaction medium. The old thesis was therefore adopted that pyridoxal-5′-phosphate is necessary for optimal aspartate aminotransferase catalytic activity; at the same time it was thought that the pyridoxal-5′-phosphate content of various control sera and standards is subject to considerable variations. Methods not including pyridoxal-5′-phosphate in the reaction medium would therefore give different results for serum and standard, compared with methods that include additional pyridoxal-5′-phosphate in the reaction medium. If a serum or standard already contains sufficient pyridoxal-5′-phosphate, then the addition of more pyridoxal-5′-phosphate will have no effect, but when a serum or standard is deficient in pyridoxal-5′-phosphate the addition of the coenzyme will alter the results.

Although many national recommendations for aspartate aminotransferase measurement include pyridoxal-5′-phosphate, addition of the cofactor is not obligatory in the German and Scandinavian recommendations. In the Scandinavian recommendations of 1974 (9) a possible supplementation with pyridoxal-5′-phosphate was suggested especially for aspartate aminotransferase. Special mention was made in that publication of the lack of pyridoxal phosphate in most commercial control sera. The use of pyridoxal-5′-phosphate is therefore not so self evident, not even in the Netherlands, where 48% of the laboratories do no include it in the reaction medium for aspartate aminotransferase determination, despite strong evidence in the literature (3–6) of the activating effect of pyridoxal-5′-phosphate on aminotransferase.

for enzymes in the Netherlands Quality Assessment programme for clinical chemical hospital laboratories, it is now possible to discriminate for temperature and also, as far as the aminotransferases

are concerned, for addition of pyridoxal-5′-phosphate.

The problem of the enzyme standard in aspartate aminotransferase determinations has been investigated in a trial of the coupled external/internal program (7) and also in the external scheme.

It was thought worthwhile to call attention once more to the problem of pyridoxal-5′-phosphate supplementation of reaction media for the measurement of aminotransferase catalytic activity, especially for the normalization of enzyme determination with standards.

Materials and Methods

In the coupled external/internal programme some 70 laboratories analysed, over a period of 40 days, Precinorm U, a bovine serum from Boehringer Mannheim lot no Ch 1-501. Omega, a human serum from Hyland, Travenol, lot no 4833 Y005 BA was used and considered as the enzyme 'standard' in this trial, although the material in fact is not a standard.

In table 1 the codes are given for the different methods used. The pyridoxal-5′-phosphate of serum and standard was measured according to the method described by *Westerhuis & Hafkenscheid* (8). In the external quality assessment programme, 4 sera were used for this investigation. Sera I and II are human sera (Ortho, lot nos. X39Y02B and X40Y02B). Serum III is a horse serum and serum IV is of human origin (Netherlands Institute of Public Health).

Tab. 1. Method codes for aspartate (ASAT) and alanine aminotransferase (ALAT) determinations.

Code		Method	Buffer	Temperature (°C)	Pyridoxal-5′-phosphate added	Reference
ASAT	ALAT					
120	130	manual	tris	25	yes	
121	131	automated	tris	25	yes	
122	132	manual	tris	25	no	
123	133	automated	tris	25	no	
124	134	manual	phosphate	25	no	10
125	135	automated	phosphate	25	no	10
126	136	centrifugal analyser	phosphate	25	no	10
127	137	centrifugal analyser	tris	30	yes	11
128	138	manual	tris	30	yes	11
129	139	automated	tris	30	yes	11
620	630	manual	tris	30	no	
621	631	automated	tris	30	no	
622	632	manual	phosphate	30	no	
623	633	automated	phosphate	30	no	
624	634	manual	tris	37	yes	
625	635	automated	tris	37	yes	
626	636	manual	tris	37	no	
627	637	automated	tris	37	no	
628	638	manual	phosphate	37	no	
629	639	automated	phosphate	37	no	

Tab. 2. Catalytic concentrations of aspartate aminotransferase in U/l in Omega and Boehringer sera. n is the number of participating laboratories. The result per laboratory is the mean of 40 independently performed analyses. Results from internal/external scheme.

Method	Boehringer serum		Omega serum		Ratio	Pyridoxal-5′-phosphate added
	n	mean	n	mean		
122	1	81.4	1	50.8	1.60	no
124	5	50.5	4	42.3	1.20	no
125	4	38.2	4	26.5	1.44	no
127	29	49.8	25	45.4	1.10	yes
128	1	50.8	1	50.2	1.01	yes
129	18	49.9	18	43.3	1.14	yes
621	2	49.0	2	32.0	1.53	no
623	2	55.4	1	29.6	1.87	no
626	1	60.1	1	33.0	1.82	no
627	3	73.9	2	43.9	1.68	no
629	2	86.9	2	58.6	1.48	no

Results

In table 2 the results are presented for the different methods used in the determination of aspartate aminotransferase with the Boehringer and Omega material. It should be remembered that the result of each laboratory is the average of 40 measurements on different days, so even method results obtained with few laboratories are relevant. In table 3 results are compared for aspartate aminotransferase and alanine aminotransferase measurement by similar methods with and without pyridoxal-5′-phosphate addition. The pyridoxal-5′-phosphate content of the sera is given in table 4. In table 5 data are presented from two trials of the external quality assessment programme.

Statistical evaluation

Table 5 shows a comparison of results obtained at the same temperature, but in the presence and absence of pyridoxal-5′-phosphate (*Student*'s t-test).

Tab. 4. Pyridoxal-5′-phosphate content of 6 sera used in this investigation. Serum III and IV were made available by RIVM (Netherlands Institute of Public Health).

	Pyridoxal-5′-phosphate (µg/l)
Omega	2.9
Boehringer	110
Serum I Ortho	11.5
Serum II Ortho	9.5
Serum III	108
Serum IV	156

For aspartate aminotransferase, the results obtained with methods 127, 128 and 129 are similar ($p > 0.8$) for both sera I and II.

Results obtained with methods 621 and 128 are not similar ($p < 0.001$).

The same holds for results found with methods 121 and 123 ($p < 0.001$) and methods 624 and 627 ($p < 0.001$).

Tab. 3. Comparison of aspartate (ASAT) and alanine aminotransferase (ALAT) results in U/l with identical methods, with the exception of pyridoxal-5′-phosphate addition (P-5′-P) to the medium. Results from internal/external scheme.

Method	Temperature (°C)	Buffer	P-5′-P	Boehringer		Omega	
				ASAT	ALAT	ASAT	ALAT
Centrifugal fast analyser	30	tris	yes	49.8	42.2	45.4	28.9
Automated	30	tris	yes	49.9	40.8	43.3	29.8
Automated	30	tris	no	49.0	39.8	32.0	26.1

Tab. 5. Aminotransferase results in U/l from the Netherlands external quality assessment programme. Serum I, II and IV are of human origin, serum III is a horse serum. Pyridoxal-5′-phosphate was added (100 µg/l) to sera III and IV.

Method code	Pyridoxal-5′-phosphate added	Temperature (°C)	Serum I		Serum II		Serum III		Serum IV	
			n	$\bar{x} \pm s$	n	$\bar{x} \pm s$	n	$\bar{x} \pm s$	n	$\bar{x} \pm s$
Aspartate aminotransferase										
127	yes	30	28	69.3 ± 3.9	43	173.6 ± 23.2	42	171.0 ± 10.3	37	25.2 ± 2.3
128	yes	30	5	69.4 ± 1.7	7	178.7 ± 9.8	7	170.4 ± 17.1	6	25.2 ± 1.5
129	yes	30	30	68.5 ± 4.2	30	176.5 ± 14.5	36	167.4 ± 7.4	40	26.0 ± 2.9
621	no	30	3	39.7 ± 0.6	4	103.5 ± 6.5	4	169.5 ± 1.3	5	28.8 ± 1.9
121	yes	25	3	40.7 ± 2.9	3	111.0 ± 11.0				
123	no	25	4	27.8 ± 1.0	6	71.8 ± 6.6				
624	yes	37	3	74.0 ± 4.6	3	196.3 ± 11.0				
627	no	37	11	59.5 ± 1.8	10	159.4 ± 7.3				
Alanine aminotransferase										
137	yes	30	34	29.6 ± 3.6	30	101.9 ± 8.6	36	42.8 ± 3.6	36	14.7 ± 1.9
138	yes	30	26	30.8 ± 3.3	18	95.9 ± 3.3	22	44.6 ± 4.8	23	16.0 ± 2.2
139	yes	30	34	30.4 ± 2.6	33	97.9 ± 6.7	33	43.3 ± 2.8	40	15.6 ± 2.5
631	no	30	5	25.2 ± 1.1	4	89.5 ± 3.4	5	41.6 ± 2.6	4	16.8 ± 1.0
131	yes	25	4	23.5 ± 5.3	4	78.3 ± 17.7				
133	no	25	5	17.8 ± 1.9	6	55.0 ± 8.4				
634	yes	37	3	33.0 ± 6.2	3	113.0 ± 14.1				
637	no	37	9	34.7 ± 1.6	11	116.6 ± 6.3				

For alanine aminotransferase, the results obtained with methods 127, 128 and 129 on the one side differ significantly from those of method 621 ($p < 0.01$).

The differences between methods 121 and 123 are significant at the 5% level ($p < 0.05$).

No significant differences were observed between results with method 624 and 627 ($p < 0.2$).

Tested for the F-distribution the variances were considered equal ($p < 0.95$).

Discussion and Conclusions

From table 2 it is obvious that 70% of the laboratories participating in the coupled external/internal programme use the aspartate aminotransferase method recommended by the NVKC (Netherlands Society for Clinical Chemistry). In contrast to this finding in the external programme, only 47% of the participants use the recommended method for aspartate aminotransferase, in which pyridoxal-5′-phosphate is added. It is also obvious that the ratio of Boehringer/Omega results is higher when pyridoxal-5′-phosphate is omitted than when it is included.

Consideration of table 3 and 4 makes it clear why the use of a standard not always improves the results of aspartate aminotransferase.

When the pyridoxal-5′-phosphate content of a sample is low, the differences in analytical results between methods including or omitting pyridoxal-5′-phosphate become obvious. However, when the sample does contain a considerable amount of pyridoxal-5′-phosphate these differences are eliminated.

Table 3 also shows the results for alanine aminotransferase, which demonstrate that pyridoxal-5′-phosphate activation of alanine aminotransferase is smaller than for aspartate aminotransferase.

Data from the external trials presented in table 5 confirm the statements made above. Again differences in aspartate aminotransferase results occur for different methods when the pyridoxal-5′-phosphate content of serum is low (tab. 5). The effect is demonstrated for different temperatures (serum I and II). In table 5 results are also given for sera containing considerable amounts of pyridoxal-5′-phosphate, and it can be seen that methodological effects then disappear (serum III and IV).

Too few data were available to show the effects for temperatures other than 30°C.

These findings seem to contrast with data from the literature (6, 8), which report similar sensitivity towards pyridoxal-5′-phosphate for both aspartate and alanine aminotransferase activity measurements.

In conclusion it can be stated that recommendations for aminotransferase catalytic activity measurement should include pyridoxal-5′-phosphate in the reaction medium to optimize specificity. Manufacturers of control sera should be advised to add pyridoxal-5′-phosphate to these sera (50 µg/l) in order to achieve comparable analytical results with optimized and deficient methods.

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